Role of natriuretic peptides and pituitary adenylate cyclase-activating polypeptide in rat thermoregulation

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PUBLICATIONS

Journal articles

I. Pataki, I.; Jászberényi, M.; Telegdy, G. Hyperthermic effect of centrally administered natriuretic peptides in the rat. Peptides 20: 193-197; 1999.

II. Pataki, I.; Adamik, Á.; Mácsai, M.; Jászberényi, M.; Telegdy, G. Pituitary adenylate cyclase-activating polypeptide induces hyperthermia in the rat. Neuropharmacology (accepted)

III. Pataki, I.; Adamik, Á.; Telegdy, G. Isatin (indole-2, 3-dione) inhibits natriuretic peptide-induced hyperthermia in rats. Peptides (accepted)

Journal abstracts

I. Pataki, I.; Adamik, Á.; Telegdy, G. Hyperthermic effect of pituitary adenylate cyclase-activating polypeptide in the rat. Folia Endocr Japon 74: 480; 1998.

II. Pataki, I.; Adamik, Á.; Telegdy, G. Inhibitory effect of isatin on natriuretic peptide-induced hyperthermia. Regul Peptides 80: 142; 1999.

Presentations

I. Pataki, I.; Telegdy, G. The effect of natriuretic peptides on rat thermoregulation. The XXXIIIrd International Congress of Physiological Sciences, St. Petersburg, Russia, 1997.

II. Pataki, I.; Adamik, Á.; Telegdy, G. The effect of pituitary adenylate cyclase-activating polypeptide on rat thermoregulation. 5th Conference of The Hungarian Neuroscience Association, Debrecen, Hungary, 1998.

III. Pataki, I.; Jászberényi, M.; Telegdy, G. Hyperthermic effect of natriuretic peptides in the rat. The Hungarian Physiology Joint Meeting, Debrecen, Hungary, 1998.

IV. Pataki, I.; Adamik, Á.; Telegdy, G. Hyperthermic effect of pituitary adenylate cyclase-activating polypeptide in the rat. The 4th International Congress of Neuroendocrinology, Kitakyushu, Japan, 1998.

V. Pataki, I,; Adamik, Á.; Jászberényi, M.; Telegdy G. Thermoregulatory actions of natriuretic peptides. Hungarian Academy of Sciences Dept. of Chemical Sciences, Session of Peptidechemistry Board, Balatonszemes, Hungary, 1999.

VI. Pataki, I.; Adamik, Á.; Telegdy, G. Inhibitory effect of isatin on natriuretic peptide-induced hyperthermia. The Hungarian Physiology Joint Meeting, Budapest, Hungary, 1999.

ABBREVIATIONS

α-MSH	α-melanocyte-stimulating hormone
ANP	atrial natriuretic peptide
AVP	arginine vasopressin
BNP	brain natriuretic peptide
CNP	C-type natriuretic peptide
CNS	central nervous system
GIP	gastric inhibitory polypeptide
GMP	guanosine monophosphate
GRF	growth hormone-releasing factor
ICV	intracerebroventricular
IL-1	interleukin-1
IL-6	interleukin-6
IM	intramuscular
IP	inptraperitoneal
LPS	lipopolysaccharide
MAO	monoamine oxidase
NSAID	non-steroid anti-inflammatory drug
PACAP	pituitary adenylate cyclase-activating polypeptide
PHI	peptide histidine isoleucine
PHV	peptide histidine valinamide
RNA	ribonucleic acid
TNF	tumor necrosis factor
TRH	thyrotropin-releasing hormone
VIP	vasoactive intestinal polypeptide

CONTENTS

1 Introduction

The ability to keep the body temperature relatively independent from environmental conditions is a fundamental part of mammalian homeostasis. Such an isolation of the living organism from diverse temperature impacts requires highly organized control mechanisms. Researches of the past few decades have come to connect these regulatory processes to the actions of certain mediators of inflammation in the central nervous system (CNS), particularly in the hypothalamus. The above-mentioned investigations were mostly aimed at the disclosure of the mechanisms that originate, maintain and terminate fever – a hyperthermic phenomenon, which has been having overriding significance in human medicine for ages – since the adequate influence on these thermoregulatory events could lead a number of diseases to a more favorable outcome.

The discovery of the regulatory characteristics of fever has guided medicine to the exploration of a number of factors that affect the body temperature through action on the CNS. Our present ideas on the bases of fever have been derived from the late 1800s, when Liebermeister introduced the view of fever as a regulated body temperature. This was based on the observations that the warmness of the febrile subject returned to its previous level after experimental cooling or warming (51). Since then, considerable efforts have been taken to clarify the mechanisms that maintain or alter temperature regulation.

It was the connection of fever to the inflammatory response that made the mediator theory a suitable approach for the investigations of thermoregulatory mechanisms. Hyperthermic effects of certain prostaglandins and also their presumable roles in the mediation of the lipopolysaccharide (LPS) –induced fever have been reported in the seventies (30, 70). Later on the investigations have expanded over the roles of some cytokines such as interleukin-1 (IL-1) (19, 77), interleukin-6 (IL-6) (40, 58) and tumor necrosis factor (TNF) (50, 75, 82) in thermoregulation together with other endogenous peptides that – besides their probable thermoregulatory actions – were known to have hormonal effects. Arginine vasopressin (AVP) (47, 53), α-melanocyte-stimulating hormone (α-MSH) (33, 74) and other endogenously produced peptides (17, 45, 64, 79, 97) that can be found in the CNS have been suspected of having thermoregulatory capacity.

Increasing evidence on a variety of neuropeptides that can affect thermoregulation indicate by now that more than a few pathways may believably participate in the central control of body temperature. The revelation of the exact modes of thermal actions of these peptide neurohormones could not only expand our knowledge over thermoregulatory mechanisms and the significance of neuropeptides in the brain but also facilitate understanding the function of the neuroendocrine system itself.

1.1 The natriuretic peptide family

The family of atrial natriuretic peptide (ANP) and related peptides currently consists of ANP, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). ANP was first discovered in rat atrial myocardial extracts (29) and was later found in the brain (49). Natriuretic peptides have been found in human cerebrospinal fluid, CNP in the highest concentration (46).

Studies investigating the primary structure of the ANP precursor showed that atrial mRNA encodes a 150-152 amino acid residue precursor molecule termed pre-pro-atrial natriuretic peptide. The first 23-25 N-terminal amino acid residues of pre-pro-ANP contain the signal peptide domain and the remaining residues comprise pro-ANP (20). Pro-ANP exhibits strong homology among various species (human, rat, mouse, dog and rabbit) (31). The circulating form of ANP in plasma consists of a smaller 3 kDa C-terminal fragment of the pro-hormone (66). The bioactive form of ANP is a 28 amino acid residue peptide that possesses a 17-residue ring structure closed by a disulphide bridge between two cysteine residues. The N- and C-terminals protrude from the ring and consist of six and five residues, respectively (Fig. 1). Active ANP has a highly conserved structure among species.

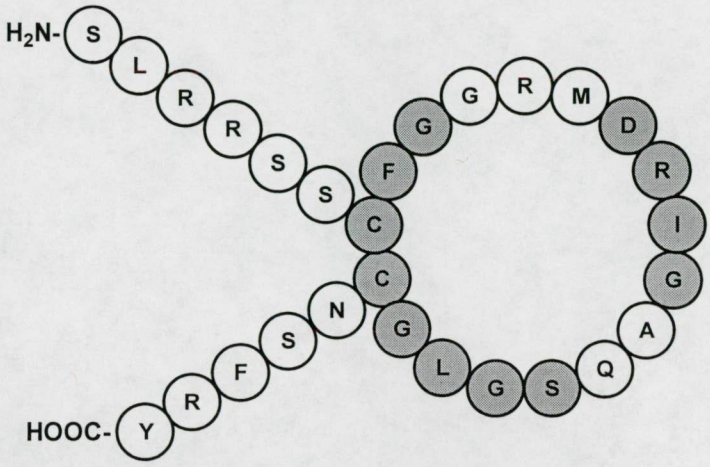

Figure 1

Structure of ANP

One letter amino acid code – A: alanine, C: cysteine, D: aspartic acid, F: phenylalanine, G: glycine, I: isoleucine, L: leucine, M: methionine, N: asparagine, Q: glutamine, R: arginine, S: serine, Y: tyrosine. Amino acid in gray circle is identical with that of BNP and CNP in the corresponding position.

Sudoh *et al.* originally isolated BNP in 1988 from porcine brain (89). BNP consists of 26 amino acids and resembles ANP in having a 17-residue ring structure closed by a disulphide bridge. A different form of BNP has been isolated from porcine brain. This contains an additional six amino acids attached at the N-terminus and has been designated BNP-32 (90). Pre-pro-BNP contains 131, while pro-BNP 106 amino acids. Both peptides have BNP at their C-terminals. An as yet uncharacterized enzyme to produce BNP and BNP-32 cleaves pro-BNP. BNP and BNP-32 are the major tissue forms of BNP in brain (54). The ring structure of BNP exhibits high homology to ANP differing by only five residues (Fig. 2).

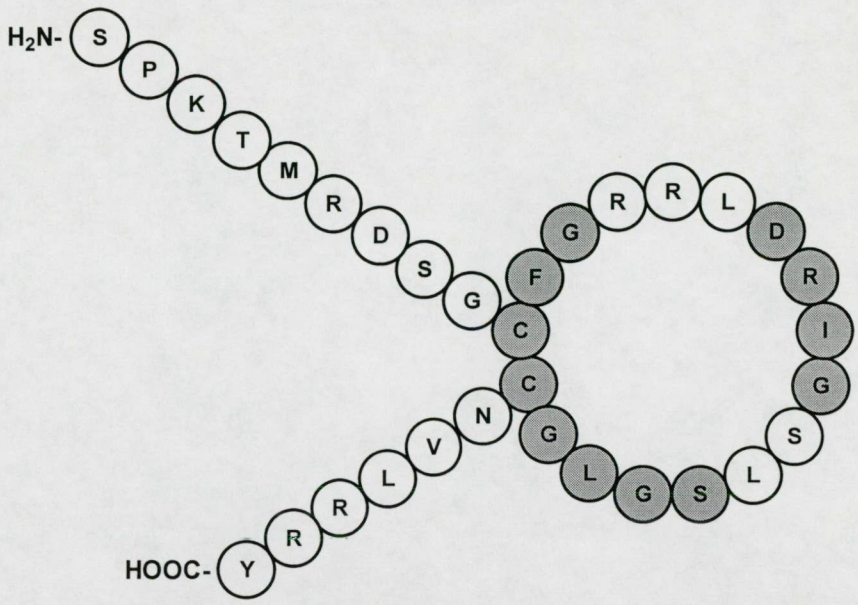

Figure 2

Structure of BNP

One letter amino acid code – A: alanine, C: cysteine, D: aspartic acid, F: phenylalanine, G: glycine, I: isoleucine, K: lysine, L: leucine, M: methionine, N: asparagine, P: proline, R: arginine, S: serine, T: threonine, V: valine, Y: tyrosine. Amino acid in gray circle is identical with that of ANP and CNP in the corresponding position.

CNP was first isolated from porcine brain in 1990 (91). CNP is present in a number of tissues, such as kidney, intestine and also in cerebrospinal fluid, but unlike ANP and BNP, has not been found in the circulation at readily detectable concentrations (66). CNP has also been detected in high concentrations in the hypothalamus, where it is assumed to function as a neuromodulator (46, 98). The pre-pro-CNP precursor molecule consists of 126 amino acid residues. Pro-CNP is a 103-residue peptide, with a 22 amino acid sequence at the C-terminus that is identical to CNP-22 (55, 95). The amino acid sequence of CNP exhibits a high degree of homology to corresponding sequences of ANP and BNP. These peptides share a 17-residue ring structure formed by an intramolecular disulphide bridge, which is characteristic of the natriuretic peptide family (66) (Fig. 3).

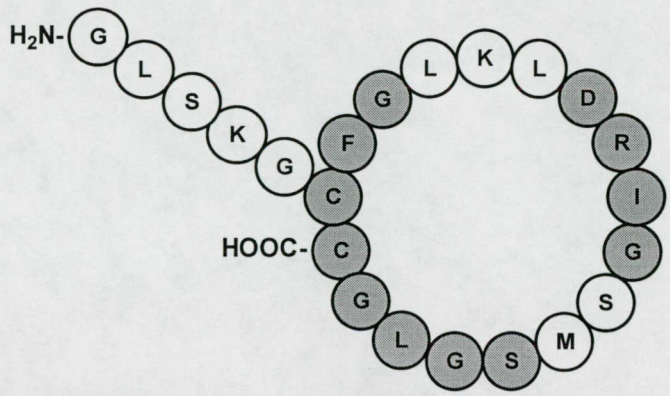

Figure 3

Structure of CNP

One letter amino acid code – C: cysteine, D: aspartic acid, F: phenylalanine, G: glycine, I: isoleucine, K: lysine, L: leucine, M: methionine, R: arginine, S: serine. Amino acid in gray circle is identical with that of BNP and CNP in the corresponding position.

The biological actions of the natriuretic peptides are mediated through association with specific, high-affinity receptors located on the surface of the target cells. Three main natriuretic peptide receptors have been cloned and named alphabetically: A, B and C (92). The A- and B-type receptors probably act via cyclic-GMP as a second messenger. The C-type receptor is less clearly linked to a second messenger system, and is presumed to be a clearance receptor (59). This latter statement about the physiological function of the C-type receptor is mainly based upon the findings that this receptor binds all the three peptides with almost equal affinity (2, 3) – although there are reports that C-type receptor can inhibit adenylate cyclase and affect phospholipase-C activity in some tissues (101), which raises the possibility that the C-type natriuretic receptor after all may have significant effect on cell function. The sequence of affinity for the A-type receptor is ANP > BNP > CNP, while that for the B-type receptor seems to be CNP > BNP > ANP (92). A new guanylyl cyclase-uncoupled natriuretic peptide receptor named D-type receptor has been recently identified in the eel in addition to the A-, B- and C-type receptor, which may help to recognize the evolutionary changes in the natriuretic peptide system (93).

1.2 Natriuretic peptides and the neuroendocrine system

Numerous studies have been undertaken to investigate the presence, distribution and localization of the natriuretic peptides and their receptors in the CNS, and it is recognized today that ANP and related peptides take part in regulatory processes in the neuroendocrine system.

Relatively little is known about the actions of these peptides on the integrated functions of the CNS. Our Department has recently carried out systemic studies to explore the roles of ANP and related peptides in learning (12, 14), behavior (13) and opiate effects (5, 6, 7). Anxiolytic properties of these peptides have also been demonstrated in an elevated plus-maze model (15, 16). Abundant modes of action of natriuretic peptides on sites in the hypothalamus and pituitary have been demonstrated (65, 73, 76, 83, 84, 85), no study, however, has yet correlated these neuropeptides with thermoregulatory processes. Although the presence of natriuretic peptides and their receptors in astrocytes has been established (52, 67, 103), no evidence exists concerning astrocytes mediating biological effects of ANP and related peptides.

1.3 Natriuretic peptides – interactions with isatin

Interactions of some members of the natriuretic peptide family with an endogenous indole, isatin (indole-2, 3-dione), were recently reported (32). Isatin is distributed throughout the CNS and is present in other tissues at levels that suggests that it has physiological effects (69); however, its origin and metabolic pathways are still uncertain. It selectively inhibits monoamine oxidase (MAO) B in vitro (32). Although its anxiogenic properties (11) and dose-related proconvulsant and anticonvulsant activities have been demonstrated in rats (8), the functional significance of these phenomena is not yet known.

Isatin and some analogues have been found to act as antagonists on natriuretic peptide receptors of the brain and heart, and the possibility has therefore arisen that isatin is an endogenous inhibitor of these peptides at a central level (68). Although antagonism of isatin against some of the behavioral effects of ANP has been described (10), the mechanisms of interaction of isatin and the natriuretic peptide system remain to be identified (69).

1.4 PACAP and its receptors

Pituitary adenylate cyclase-activating polypeptide (PACAP) is known as a member of a superfamily that includes vasoactive intestinal polypeptide (VIP), secretin, glucagon, peptide histidine isoleucine (PHI), peptide histidine valinamide (PHV), helodermin, helospectin, gastric inhibitory polypeptide (GIP) and growth hormone-releasing factor (GRF) (23) (Table 1). PACAP was originally isolated from the ovine hypothalamus (71). The biologically active neuropeptide exists in two amidated forms: PACAP-38, a 38-amino-acid polypeptide; and PACAP-27, a truncated form of PACAP-38 containing 27 residues (72). It now appears that, in all tissues, PACAP-27 represents only a minor portion of total PACAP. The extreme conservation of the structure of PACAP in vertebrates means that this neuropeptide is subjected to strong evolutionary pressure and may play a substantial role (23). Both PACAP-38 and PACAP-27 are potent in stimulating adenylate cyclase.

At least two receptor classes have been reported for PACAP in mammalian tissues: type I and type II. Type I receptors are highly selective in the recognition of PACAP much more potently than VIP, but type II receptors display similar high affinity for PACAP-27, PACAP-38 and VIP (21). In the CNS, type I PACAP receptors are abundant whereas the amount of type II receptors is low (36). Type I receptors can be divided into two, and type II receptors into three subtypes (23). The PACAP-A subtype of the type I receptor exhibits hardly any preference for PACAP-38 over PACAP-27, whereas the PACAP-B subtype recognizes PACAP-38 with high affinity and PACAP-27 with low affinity.

Type II (VIP-PACAP) receptors almost exclusively interact with adenylate cyclase. At least two effector systems, however, exist for type I PACAP-preferring receptors (23). It appears that type I receptors stimulate both adenylate cyclase and phospholipase C, this

coupling to dual signaling cascades involving interactions with G proteins of the G_s and G_q types. Subsequent to phospholipase C activation and through the inositol phosphate cascade, a secondary Ca^{2+} entry elevating cytosolic [Ca^{2+}] follows Ca^{2+} mobilization (23).

Species	Peptide	Amino acid sequence
o/h/r	PACAP-38	H-S-D-G-I-F-T-D-S-**Y**-S-R-Y-R-K-Q-M-A-V-**K**-K-Y-L-A-A-V-L-G-K-**R**-Y-K-Q-R-V-K-N-K-NH_2
o/h/p/f	PACAP-27	H-S-D-G-I-F-T-D-S-**Y**-S-R-Y-R-K-Q-M-A-V-**K**-K-Y-L-A-A-V-L-NH_2
b/do/go/h/o/p/r/ra	VIP	H-S-D-A-V-F-T-D-N-**Y**-T-R-L-R-K-Q-M-A-V-**K**-K-Y-L-N-S-I-L-N-NH_2
b/p/gp/o	secretin	H-S-D-G-T-F-T-S-E-**L**-S-R-L-R-D-S-A-R-L-**Q**-R-L-L-Q-G-R-V-NH_2
b/h/p	glucagon	H-S-Q-G-T-F-T-S-D-**Y**-S-K-Y-L-D-S-R-R-A-**Q**-D-F-V-Q-W-L-M-N-T
p	GIP	Y-A-E-G-T-F-I-S-D-**Y**-S-I-A-M-D-K-I-R-Q-**Q**-D-F-V-N-W-L-L-A-Q-**K**-G-K-K-S-D-W-K-H-N-**I**-T-Q
p	GRF (1-44)	Y-A-D-A-I-F-T-N-S-**Y**-R-K-V-L-G-Q-L-S-A-**R**-K-L-L-Q-D-I-M-S-R-**Q**-Q-G-E-R-N-Q-E-Q-G-**A**-R-V-R-L-NH_2

Table 1

Amino acid sequence of PACAP and some related peptides

One letter amino acid code – A: alanine, C: cysteine, D: aspartic acid, F: phenylalanine, G: glycine, H: histidine, I: isoleucine, K: lysine, L: leucine, M: methionine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, W: threonine, Y: tyrosine.

Species: b: bovine, do: dog, f: frog:, go: goat, gp: guinea pig, h: human, o: ovine, p: porcine, r: rat, ra: rabbit

1. 5 PACAP and the neuroendocrine system

The heterogeneous distribution of PACAP in the rat central nervous system (CNS) has been demonstrated (26). The peptide has been observed in several nuclei of the hypothalamus, thalamus, ventral tegmental area, septum, amygdala and brainstem (63). PACAP-specific

binding sites are found throughout the rat brain (62). The presence of PACAP receptors has also been demonstrated in astrocytes (4, 94); however, their physiological importance is not clear.

The locations of PACAP and its receptors suggest that this peptide may be involved in regulatory processes in the neuroendocrine system. Relatively little is known about the action of PACAP on the integrated functions of the CNS. Although abundant information is available regarding the action of PACAP on sites in the pituitary and the hypothalamus (43, 56, 102), it appears that PACAP variously affects hormone release from anterior pituitary depending on the cell type and experimental conditions. Dissenting evidence exist on the effects of PACAP on the secretion of pituitary hormones in *in vitro* static (25, 71), dynamic (34) and *in vivo* systems (37). In a number of cases the dose-response curve of PACAP seemed bell-shaped (maybe due to desensitization at high doses) (23, 71). Delayed hypopthalamo-hypophiseal actions in response to PACAP may reflect the interaction of a second messenger, induction of an additional transduction signal and/or effects on transcription (23, 43).

Only a few studies have yet correlated this neuropeptide with thermoregulatory processes. It has been reported that PACAP counteracted reserpine-induced hypothermia in mice. Injections of PACAP-38 dramatically reversed the body temperature lowered by reserpine, and it was suggested that PACAP might have an important temperature-regulating function in the CNS (61).

The above-mentioned observation prompted us to investigate the central effects of PACAP on body temperature.

1.6 The aims of our experiments

Our experiments were designed to study

1. the effects of ANP and related peptides on the body temperature of rats;
2. the comparison of the hyperthermic effects of the most efficacious doses of different natriuretic peptides in rats;

3. the effects of noraminophenazone on the natriuretic peptide-induced hyperthermia in rats;
4. the effects of isatin on the natriuretic peptide-induced hyperthermia in rats;
5. the effects of PACAP-38 on the body temperature of rats;
6. the effects of PACAP-38 antiserum on the body temperature and on the hyperthermic effect of PACAP-38 in rats;
7. the effects of noraminophenazone on the PACAP-38-induced hyperthermia in rats.

2 Materials and methods

2.1 Materials

The materials used in the experiments were of the following origins:

- Sterile pyrogen-free saline (Natr. chlor. inj. 0.9 %, Biogal, Hungary)
- Normal rabbit serum (this was a honorous gift from Dr. Miklós Vecsernyés, Department of Endocrinology Albert Szent-Györgyi Medical University, Szeged, Hungary)
- Sodium pentobarbital (Nembutal, CEVA, France)
- ANP-28, BNP-32 and CNP-22 (Bachem California Inc., Torrance CA, USA)
- Noraminophenazone (Algopyrin, Chinoin, Hungary)
- Isatin (indole-2, 3-dione, Sigma Chemical Co., St. Louis MO, USA)
- PACAP-38 (Bachem, Switzerland)
- Human PACAP-38 antiserum (PACAP-38-AB, Yanaihara Institute Inc. Japan)

2.2 Animals

All experiments were performed on adult male Wistar rats weighing 200 ± 30 g. The animals were kept in a room maintained at constant temperature (23 ± 1 °C) and on a 12-h dark-light cycle (lights on from 6:00 to 18:00). Commercial laboratory food and tap water were given *ad libitum*. At least a week of recovery from surgery was allowed before the beginning of the experiments. Each animal was used only once in the experiments.

The animals were kept and handled during the experiments in accordance with the instructions of Albert Szent-Györgyi Medical University Ethical Committee for the Protection of Animals in Research.

2.3 Surgery

In order to allow intracerebroventricular (ICV) peptide administration, the rats were implanted with a cannula introduced into the right lateral brain ventricle before the

experiments. Under pentobarbital (Nembutal, 35 mg/kg), intraperitoneal (IP) anesthesia, the stainless steel cannula was stereotaxically inserted into the ventricle with coordinates 0.2 mm posterior; 1.7 mm lateral to the bregma; 3.7 mm deep from the dural surface according to the atlas of Pellegrino et al. (80). The cannula was secured with dental acrylic cement. Rats were allowed a minimum of 5 days to recover from surgery before peptide treatment. The correct positioning of the cannula was checked by injecting 10 μl methylene blue into the ventricle of decapitated animals through the cannula and subsequent dissection of the brain. Animals with incorrect placement of the cannula were discarded and excluded from the statistical evaluations.

2.4 Measuring-instruments

In experiments with natriuretic peptide-effects on the body temperature a digital electric thermometer (Model: Thermini 130) whereas in all other experiments a digital electric thermometer (Model: Cole-Parmer 8402-10) was used to monitor the colon temperature.

2.5 Treatments

2.5.1 Effects of natriuretic peptides on colon temperature

For ICV treatment, different doses of ANP-28, BNP-32 or CNP-22 were dissolved in sterile pyrogen-free 0.9% saline and injected in a volume of 2 μl into the lateral brain ventricle of the animals.

A pyrazolone derivative noraminophenazone was used to inhibit cyclooxygenase. Noraminophenazone was administered intramuscularly (IM) in a dose of 50 mg/kg, diluted in saline in a volume of 2.5 ml/kg.

The control animals received the same volume of saline for all experiments.

2.5.2 Effects of isatin on natriuretic peptide-induced hyperthermia

Treatments with ANP-28, BNP 32 and CNP-22 were similar to the above described except that in accordance with our previous experience (78), only the most effective dose of the natriuretic peptides (1 μg) was selected. Isatin was administered IP in a dose of 50 mg/kg,

diluted in saline in a volume of 2.5 ml/kg. The control animals received the same volume of saline for all experiments.

2.5.3 Effects of PACAP on colon temperature

For ICV treatment, different doses of PACAP-38 were dissolved in sterile pyrogen-free 0.9% saline and injected in a volume of 2 µl. The control groups were treated with saline for all experiments.

Noraminophenazone was administered intramuscularly (IM) in a dose of 50 mg/kg, diluted in saline in a volume of 2.5 ml/kg.

PACAP-38-AB was diluted with saline. The specificity of the PACAP-38-AB was as follows: PACAP-38 100%, PACAP-38 (5-38) 100%, PACAP-38 (10-38) 57.2%, PACAP-38 (16-38) 173%, PACAP-38 (22-38) 333%, PACAP-27 < 0.1%, glucagon 0%, VIP 0%, PHI 0%, secretin 0%. Different dilutions (1:5, 1:10 and 1:20) of PACAP-38-AB were injected ICV in a volume of 2 µl. In experiments with PACAP-38-AB, the control animals received the same volume of normal rabbit serum, in the respective dilutions.

2.6 Procedures

In order for the rats to habituate to the experimental environment, the animals were transferred to the laboratory 2 h before the beginning of the test, on the day of the experiment. The animals were kept in the laboratory at constant temperature (23.0 ± 0.5 °C) before and throughout the experiment. Each animal was removed from the cage and gently restrained on the table with a cloth. The colon temperature was monitored by inserting a vaseline-lubricated thermistor probe of a digital electric thermometer 5 cm into the rectum of the animal. The probe was inserted the same distance into the rectum each time it was used.

The following experiments were performed:

2.6.1 Effects of natriuretic peptides on colon temperature

All experiments started at 8 a.m. with an initial colon temperature measurement, the colon temperature being measured before and 30, 60 and 90 min after peptide treatment.

1. The effects of centrally administered ANP-28, BNP-32 and CNP-22 on body temperature were studied. The natriuretic peptides were injected ICV in different doses (40-1000 ng).
2. In studies with noraminophenazone on the hyperthermic effect of the natriuretic peptides, noraminophenazone was administered IM once 30 min before ICV peptide treatment (ANP-28, BNP-32 or CNP-22 being injected in doses of either 400 ng or 1000 ng) and the colon temperature was measured 30, 60 and 90 min later.

2.6.2 Effects of isatin on natriuretic peptide-induced hyperthermia

All experiments started at 8 a.m. with an initial colon temperature measurement, the colon temperature being measured before and 30, 60 and 90 min after peptide treatment.

1. The effects of centrally administered ANP-28, BNP-32 and CNP-22 on body temperature were studied. The natriuretic peptides were injected ICV in a dose of 1 μg.
2. In studies with isatin on the hyperthermic effects of natriuretic peptides, isatin (50 mg/kg; IP) was administered once 30 min before ICV peptide treatment (ANP-28, BNP-32 or CNP-22 being injected in a dose of 1 μg) and the colon temperature was measured 30, 60 and 90 min later.

2.6.3 Effects of PACAP on colon temperature

The experiments started at 8 a.m. with an initial colon temperature measurement, the colon temperature also being measured before and 1, 2, 3, 4, 5, 6 and 24 h after peptide treatment. In studies with PACAP-38-AB, colon temperature was measured before and 1, 2, 3, 4, 5, 6, 7, 8 and 9 h after PACAP-38-AB treatment.

1. The effects of centrally administered PACAP-38 on body temperature were studied. The peptide was injected ICV in different doses (250 ng/55 pmol, 500 ng/110 pmol and 1000 ng/220 pmol), and the colon temperature was measured every hour for 6 h and at 24 h after PACAP treatment.
2. The effects of centrally administered PACAP-38-AB on body temperature and on PACAP-induced hyperthermia were studied. PACAP-38-AB was dissolved in physiological saline in dilutions of 1:5, 1:10 and 1:20. Two μl was administered ICV 3 h before PACAP-38 (1000 ng, ICV) or saline (ICV) injection. To ascertain the possible effects of rabbit serum, groups of animals received normal rabbit serum in the same dilutions and volumes as the

PACAP-38-AB 3 h before either ICV PACAP-38 or saline injections. Colon temperature was measured every hour for 9 h starting from PACAP-38-AB treatment.

3. In studies with noraminophenazone on the hyperthermic effect of PACAP-38, noraminophenazone was administered IM once 30 min before ICV peptide treatment (PACAP-38 being injected in a dose of 1000 ng) and the colon temperature was measured 1, 2, 3, 4, 5 and 6 h later.

2.7 Statistical analysis

Statistical analysis of the data was made by analysis of variance (ANOVA). For significant interactions (time×treatment) with two-way ANOVA, one-way ANOVA was performed, and then groups were compared by Tukey's post hoc test for multiple comparisons with unequal cell size. A probability level of 0.05 was accepted as indicating significant differences.

3 Results

3.1 The effects of ANP-28, BNP-32 and CNP-22 on body temperature

3.1.1 Hyperthermic effects of different doses of natriuretic peptides

The effects of different doses of natriuretic peptides on the colon temperature were measured after ICV injections of dose-series of ANP-28, BNP-32 or CNP-22. 400 ng (131 pmol) and 1000 ng (327 pmol) doses of ANP-28 had a hyperthermic effect 30 min [$F(3, 33) = 40.56$; $p < 0.05$], 60 min [$F(3, 33) = 77.94$; $p < 0.05$] and 90 min [$F(3, 33) = 15.56$; $p < 0.05$] after ICV administration, the magnitude of the effect being smaller at 90 min than at 60 min. ANP-28 in a dose of 40 ng (13.1 pmol) increased the colon temperature only at 60 min (Fig. 4).

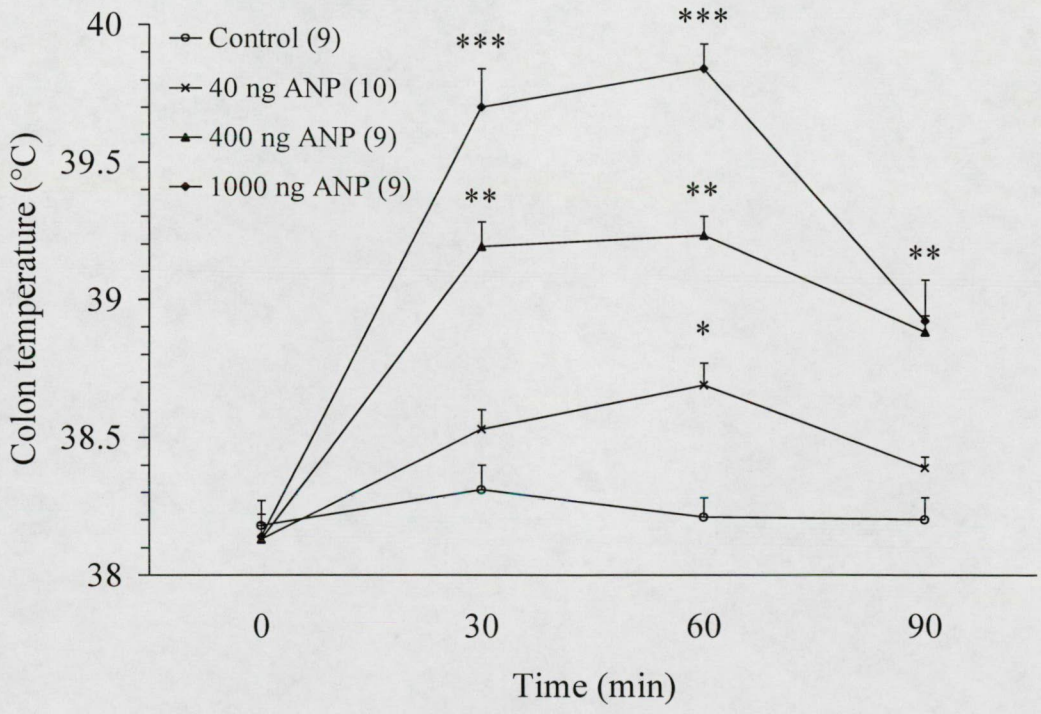

Figure 4

The effects of atrial natriuretic peptide (ANP) on colon temperature

ANP groups of rats ($n \geq 9$) received an injection of ANP-28 (40, 400 or 1000 ng, ICV). The control group received an ICV injection of saline. Number of animals per group is presented in parentheses after the

corresponding group. The vertical lines denote the S.E.M. * $p < 0.05$, compared with the control; ** $p < 0.05$, compared with the control and with the 40 ng ANP group; *** $p < 0.05$, compared with the control and with the 40 and 400 ng ANP groups.

Both 400 (11.6 pmol) and 1000 ng (290 pmol) BNP-32 increased the colon temperature at 30 min [$F(3, 53) = 32.63$; $p < 0.05$], 60 min [$F(3, 53) = 41.83$; $p < 0.05$] and 90 min [$F(3, 53) = 6.5$; $p < 0.05$] (Fig. 5).

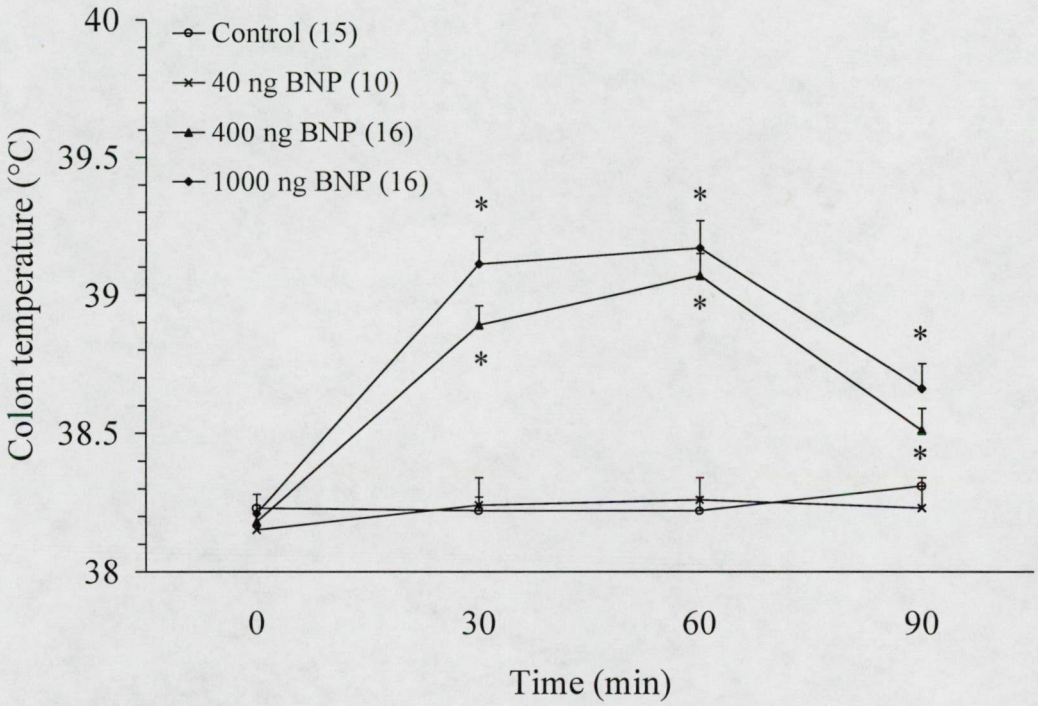

Figure 5

The effects of brain natriuretic peptide (BNP) on colon temperature

BNP groups of rats ($n \geq 10$) received an injection of BNP-32 (40, 400 or 1000 ng, ICV). The control group received an ICV injection of saline. Number of animals per group is presented in parentheses after the corresponding group. The vertical lines denote the S.E.M. * $p < 0.05$, compared with the control and with the 40 ng BNP group.

CNP-22 in doses of 400 ng (182 pmol) and 1000 ng (455 pmol) was hyperthermic both 30 min [$F(3, 34) = 15.72$; $p < 0.05$] and 60 min [$F(3, 34) = 28.48$; $p < 0.05$] after ICV administration. The 1000 ng dose of CNP-22 was also hyperthermic at 90 min [$F(3, 34) =$

4.54; $p < 0.05$]. A 40 ng (18.2 pmol) dose of CNP-22 had a mild, but statistically not significant hyperthermic effect (Fig. 6).

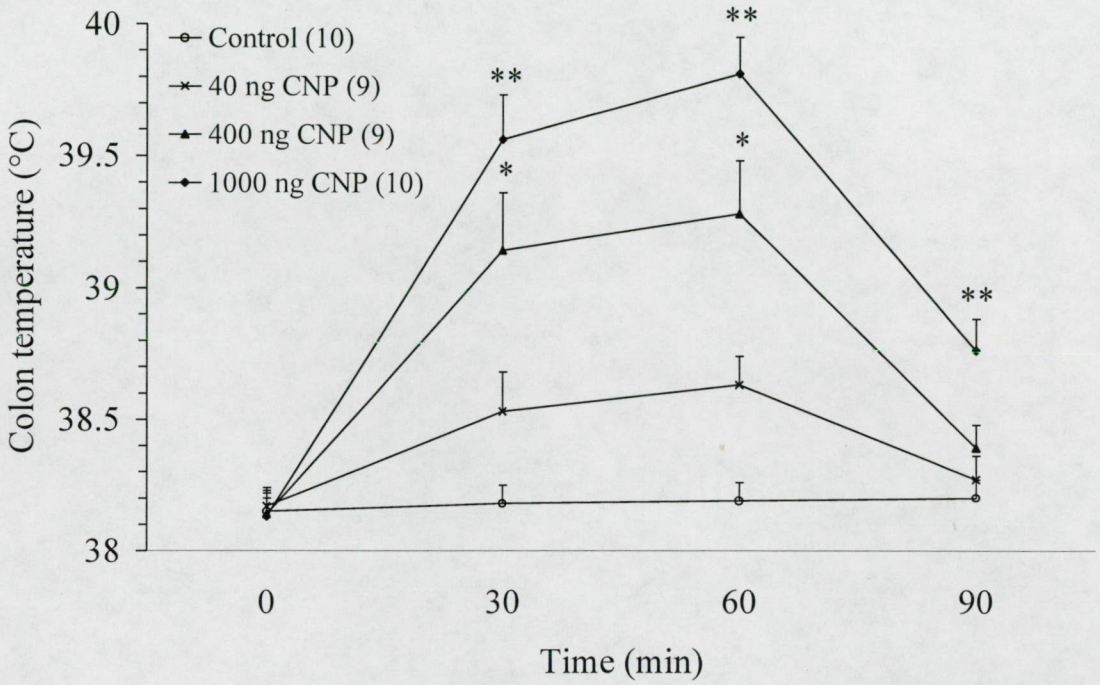

Figure 6

The effects of C-type natriuretic peptide (CNP) on colon temperature

CNP groups of rats ($n \geq 9$) received an injection of CNP-22 (40, 400 or 1000 ng, ICV). The control group received an ICV injection of saline. The vertical lines denote the S.E.M. * $p < 0.05$, compared with the control and with the 40 ng CNP group; ** $p < 0.05$, compared with the control and with the 40 and 400 ng CNP groups.

3.1.2 Comparison of the hyperthermic effects of the most efficacious doses of natriuretic peptides

Hyperthermic effects of ICV injections of a 1 μg dose of ANP-28, BNP-32 and CNP-22 were compared. A 1 μg dose of either ANP-28, BNP-32 or CNP-22 had hyperthermic effects 30 min [$F(3, 47) = 18.26$; $p < 0.01$], 60 min [$F(3, 47) = 20.36$; $p < 0.01$] and 90 min [$F(3, 47) = 10.61$; $p < 0.05$] after ICV administration, the magnitude of the effect being smaller at 90 min than at 30 and 60 min. The probability level of the statistically significant

differences as compared to the control group were lower ($p < 0.01$) for ANP-28 and CNP-22 than for BNP-22 ($p < 0.05$) at 90 min, but no statistically significant difference could be demonstrated for BNP-22 as compared to the ANP-28 and CNP-22 groups at any time point (Fig. 7). These observations were in accordance with our previously published data (78).

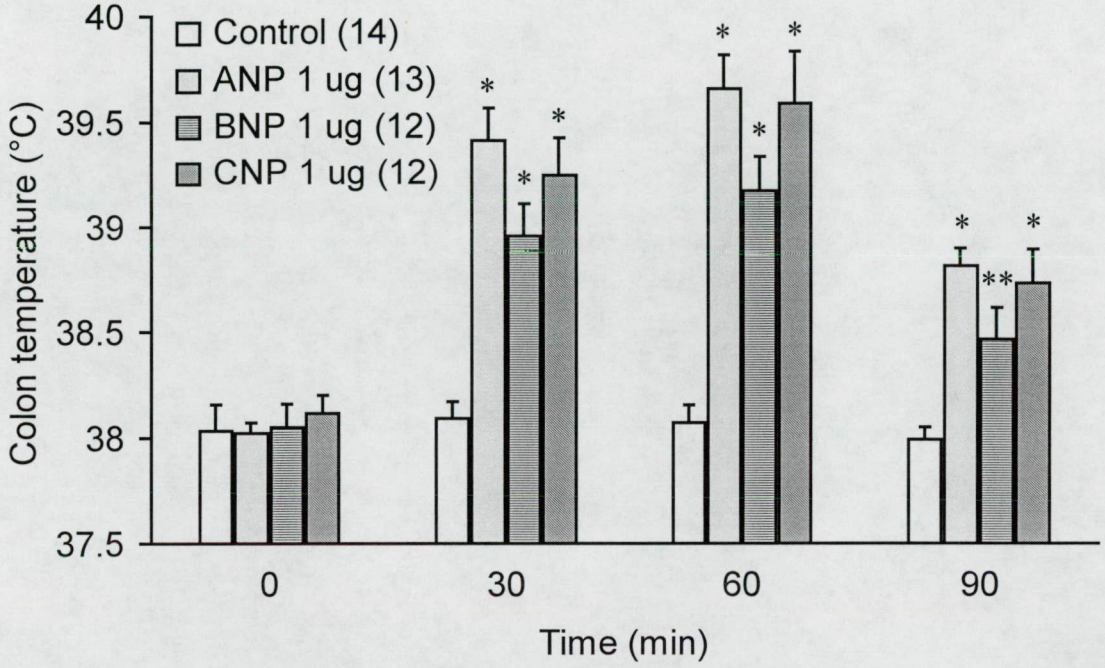

Figure 7

The effects of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) on colon temperature

Groups of rats ($n \geq 12$) received an injection of ANP-28, BNP-32 or CNP-22 (1 μg, ICV). The control group received an ICV injection of saline. Number of animals per group is presented in parentheses after the corresponding group. The vertical lines on the top of the bars denote the S.E.M. * $p < 0.01$, compared with the control; ** $p < 0.05$, compared with the control.

3.2 The effects of noraminophenazone on the natriuretic peptide-induced hyperthermia

The possible involvements of cyclooxygenase products in the natriuretic peptide-induced hyperthermia were investigated. A 50 mg/kg IM injection of the cyclooxygenase

inhibitor noraminophenazone 30 min before peptide administration abolished the hyperthermic effects of 400 and 1000 ng ANP-28 [$F_{30;60;90\ min}(5, 44) = 27.94; 43.30; 7.52; p < 0.05$] (Fig. 8), BNP-32 [$F_{30;60;90\ min}(5, 44) = 21.05; 32.25; 3.11; p < 0.05$] (Fig. 9) and CNP-22 [$F_{30;60;90\ min}(5, 45) = 49.25; 113.18; 0.91; p < 0.05$] (Fig. 10). Noraminophenazone alone in a dose of 50 mg/kg IM did not affect the body temperature.

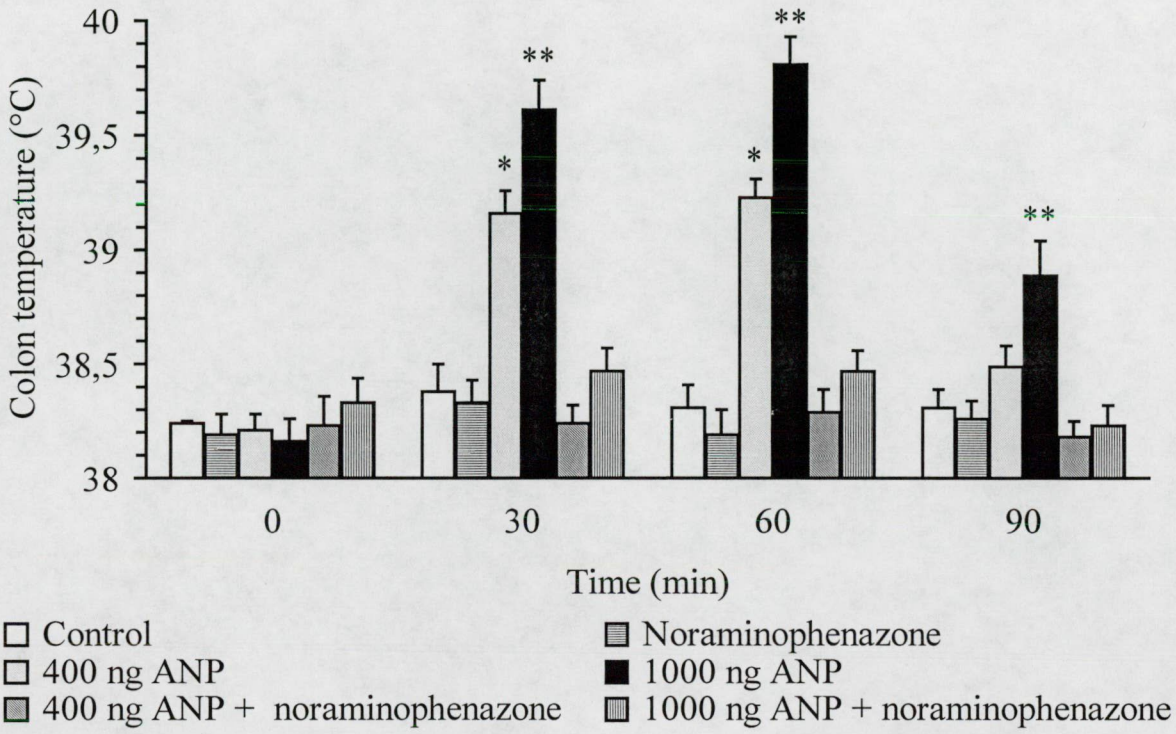

Figure 8

Inhibitory effect of noraminophenazone on ANP-induced hyperthermia

Groups of rats ($n \geq 8$) received an IM injection of saline (ANP groups) or 50 mg noraminophenazone (ANP + noraminophenazone groups) 30 min before an ICV injection of 400 or 1000 ng ANP-28. The noraminophenazone group received an ICV saline injection 30 min after noraminophenazone pretreatment (50 mg, IM). The control group received an IM saline injection 30 min before saline treatment (ICV). The vertical lines at the top of the bars denote the S.E.M. * $p < 0.05$, compared with the control and with the noraminophenazone, the 400 ng ANP + noraminophenazone and the 1000 ng ANP + noraminophenazone groups; ** $p < 0.05$, compared with the control and with the noraminophenazone, the 400 ng ANP, the 400 ng ANP + noraminophenazone and the 1000 ng ANP + noraminophenazone groups.

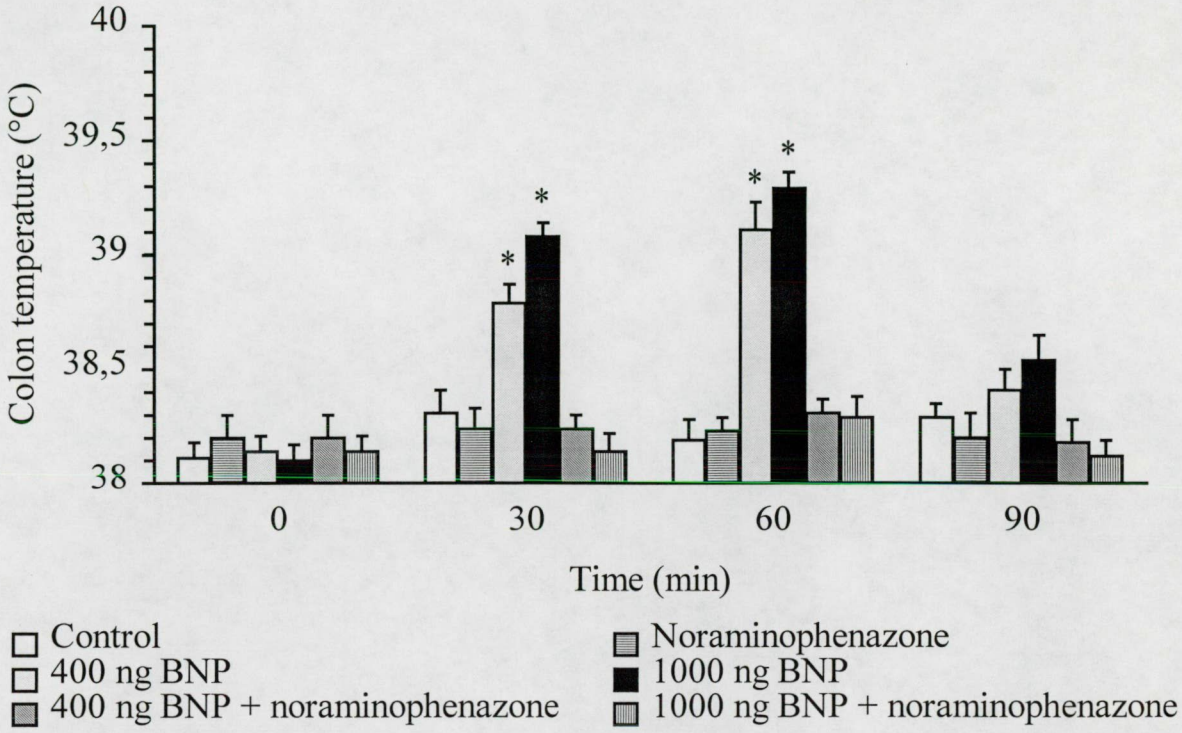

Figure 9

Inhibitory effect of noraminophenazone on BNP-induced hyperthermia

Groups of rats ($n \geq 8$) received an IM injection of saline (BNP groups) or 50 mg noraminophenazone (BNP + noraminophenazone groups) 30 min before an ICV injection of 400 or 1000 ng BNP-32. The noraminophenazone groups received an ICV saline injection 30 min after noraminophenazone pretreatment (50 mg, IM). The control group received an IM saline injection 30 min before saline treatment (ICV). The vertical lines at the top of the bars denote the S.E.M. * $p < 0.05$, compared with the control and with the noraminophenazone, the 400 ng BNP + noraminophenazone and the 1000 ng BNP + noraminophenazone groups.

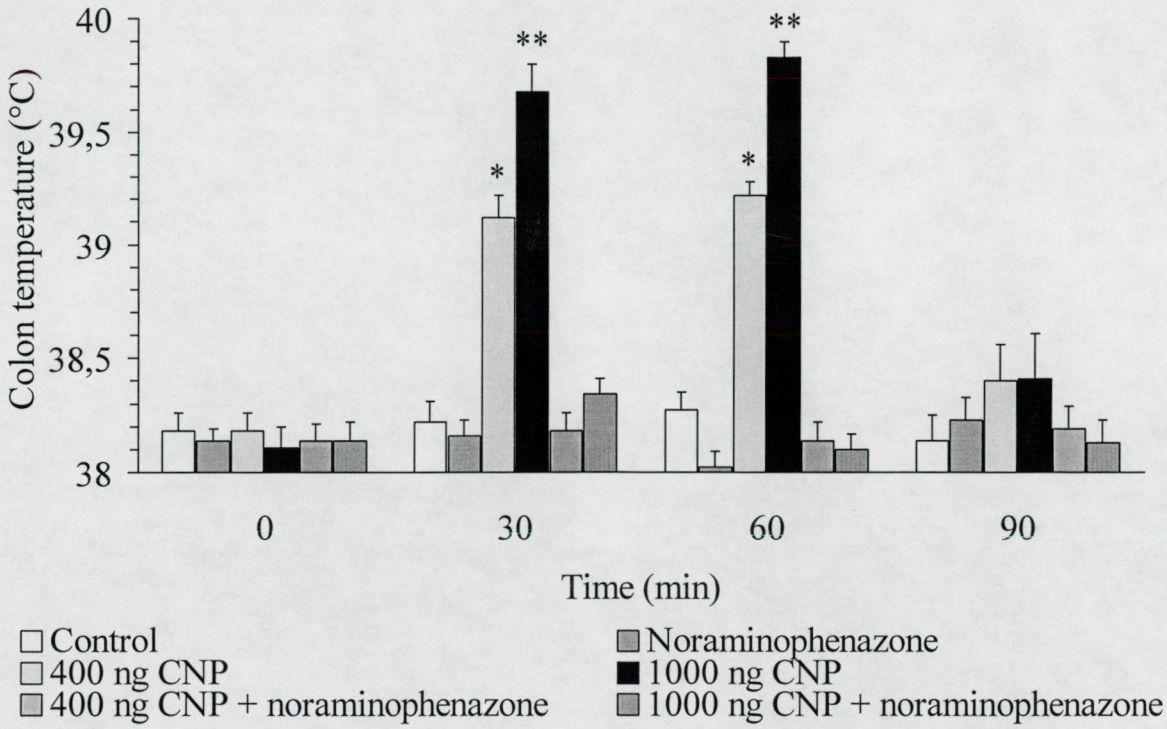

Figure 10

Inhibitory effect of noraminophenazone on CNP-induced hyperthermia

Groups of rats ($n \geq 8$) received an IM injection of saline (CNP groups) or 50 mg noraminophenazone (CNP + noraminophenazone groups) 30 min before an ICV injection of 400 or 1000 ng CNP-22. The noraminophenazone group received an ICV saline injection 30 min after noraminophenazone pretreatment (50 mg, IM). The control group received an IM saline injection 30 min before saline treatment (ICV). The vertical lines at the top of the bars denote the S.E.M. * $p < 0.05$, compared with the control and with the noraminophenazone, the 400 ng CNP + noraminophenazone and the 1000 ng CNP + noraminophenazone groups; ** $p < 0.05$, compared with the control and with the noraminophenazone, the 400 ng CNP, the 400 ng CNP + noraminophenazone and the 1000 ng CNP + noraminophenazone groups.

3.3 The effects of isatin on the natriuretic peptide-induced hyperthermia

A 50 mg/kg IP injection of the endogenous indole isatin 30 min before peptide administration abolished the hyperthermic effects of 1 μg ANP-28 [$F_{30;60;90\ min}(3, 34) = 25.39$;

50.15; 13.93; $p < 0.01$] (Fig. 11), BNP-32 [$F_{30;60\ min}(3, 38) = 17.05; 21.58; p < 0.01$], [$F_{90\ min}(3, 38) = 4.75; p < 0.05$](Fig. 12) and CNP-22 [$F_{30;60;90\ min}(3, 37) = 18.64; 23.02; 11.80; p < 0.01$] (Fig. 13). Isatin alone in a dose of 50 mg/kg IP did not affect the body temperature.

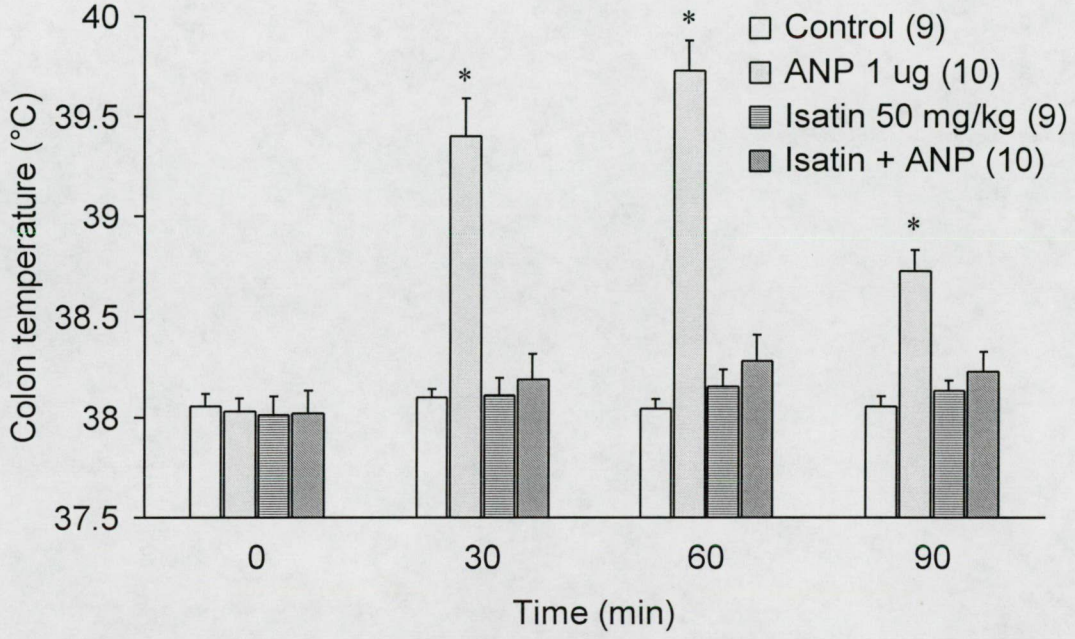

Figure 11

Inhibitory effect of isatin on ANP-induced hyperthermia

Groups of rats ($n \geq 9$) received an IP injection of saline (ANP group) or 50 mg isatin (isatin + ANP group) 30 min before an ICV injection of 1 μg ANP-28. The isatin group received an ICV saline injection 30 min after isatin pretreatment (50 mg, IP). The control group received an IP saline injection 30 min before saline treatment (ICV). Number of animals per group is presented in parentheses after the corresponding group. The vertical lines at the top of the bars denote the S.E.M. * $p < 0.01$, compared with the control, the isatin and with the isatin + ANP groups.

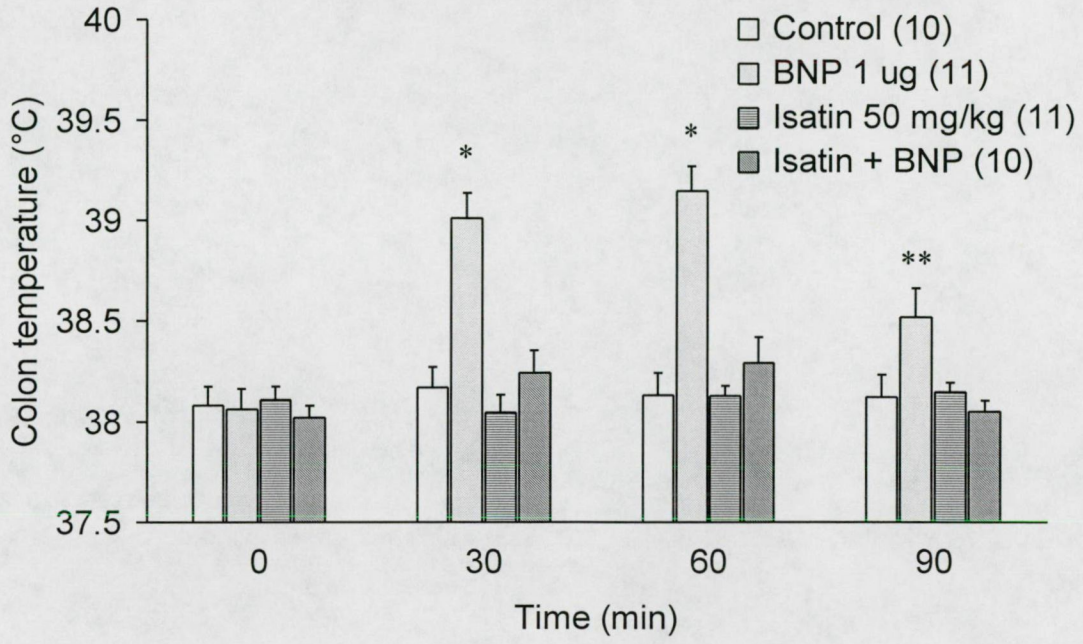

Figure 12

Inhibitory effect of isatin on BNP-induced hyperthermia

Groups of rats ($n \geq 10$) received an IP injection of saline (BNP group) or 50 mg isatin (isatin + BNP group) 30 min before an ICV injection of 1 μg BNP-32. The isatin group received an ICV saline injection 30 min after isatin pretreatment (50 mg, IP). The control group received an IP saline injection 30 min before saline treatment (ICV). Number of animals per group is presented in parentheses after the corresponding group. The vertical lines at the top of the bars denote the S.E.M. * $p < 0.01$, compared with the control, the isatin and with the isatin + BNP groups; ** $p < 0.05$, compared with the control, the isatin and with the isatin + BNP groups.

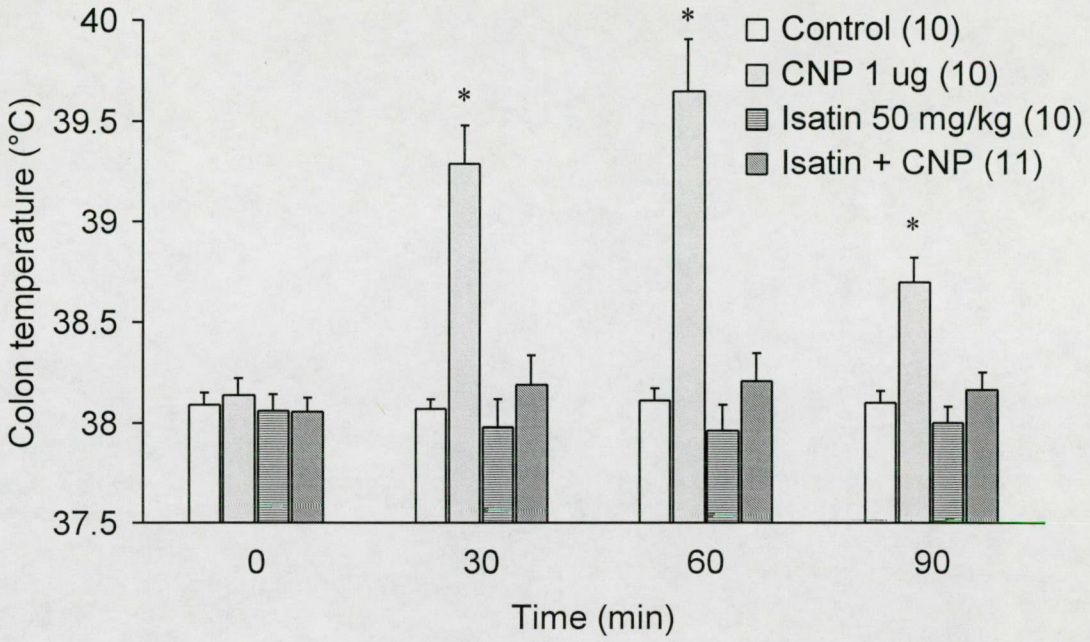

Figure 13

Inhibitory effect of isatin on CNP-induced hyperthermia

Groups of rats ($n \geq 10$) received an IP injection of saline (CNP group) or 50 mg isatin (isatin + CNP group) 30 min before an ICV injection of 1 μg CNP-22. The isatin group received an ICV saline injection 30 min after isatin pretreatment (50 mg, IP). The control group received an IP saline injection 30 min before saline treatment (ICV). Number of animals per group is presented in parentheses after the corresponding group. The vertical lines at the top of the bars denote the S.E.M. * $p < 0.01$, compared with the control, the isatin and with the isatin + CNP groups.

3.4 The effects of PACAP-38 on body temperature and the effects of PACAP-38-AB on colon temperature and on the PACAP-38-induced hyperthermia

3.4.1 The effects of PACAP-38 on body temperature

Both 500 and 1000 ng doses of PACAP-38 had hyperthermic effects 3, 4, 5 and 6 h [$F_{3;4;5;6\ h}$ (3, 38) = 25.85; 33.69; 24.38; 34.54; $p < 0.05$] after ICV administration. The 1000 ng dose of PACAP-38 was significantly more potent in increasing the colon temperature than the lower dose. There was a significant rise in colon temperature 2 h after the 1000 ng injection of

PACAP-38 [F (3, 38) = 3.31; $p < 0.05$]. A moderate decrease in the colon temperature was observed at 6 h in the PACAP-38-treated (500 or 1000 ng, ICV) animals. A 250 ng dose of PACAP-38 had no effect (Fig. 14). No change in body temperature compared to the initial values could be observed in any group 24 h after the administration of PACAP-38.

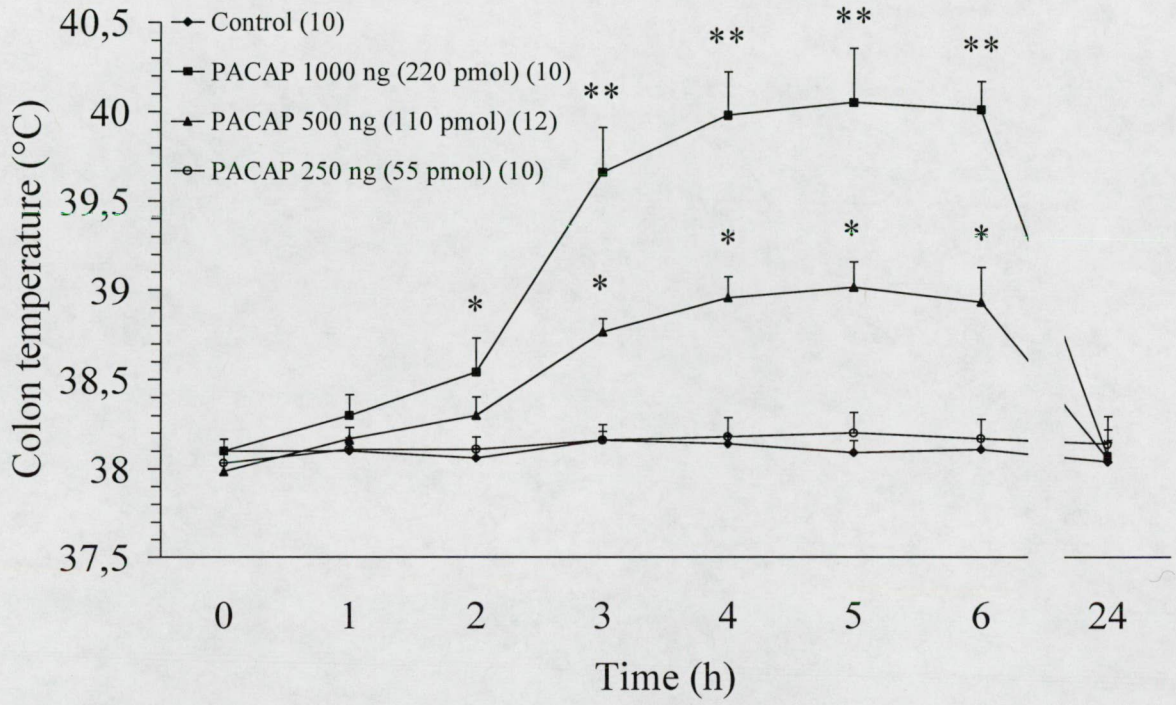

Figure 14

Effects of PACAP-38 on colon temperature

PACAP groups of rats received an injection of PACAP-38 (250, 500 or 1000 ng, ICV). The control group received an ICV injection of saline. Number of animals per group is presented in parentheses after the corresponding group. The vertical lines on the top of the marks denote the S.E.M. * $p < 0.05$, compared with the control; ** $p < 0.05$, compared with the control and with the PACAP 500 ng group.

3.4.2 The effects of PACAP-38-AB on colon temperature and on the PACAP-38-induced hyperthermia

None of the three dilutions of PACAP-38-AB (1:5, 1:10 and 1:20) modified the colon temperature of rats when given ICV 3 h before ICV saline injection (data not shown). Central

injections of PACAP-38-AB in dilutions of 1:5 and 1:10 3 h before PACAP-38 administration (1000 ng, ICV) completely abolished the hyperthermic effect of PACAP-38 [$F_{1;\,2;\,3;\,4;\,5;\,6;\,7;\,8;\,9\,h}$ (8, 58) = 1.26, 0.62, 0.42, 2.42, 9.16, 45.30, 99.77, 68.30, 63.48]. In accordance with expectations, ICV injections of the different (1:5, 1:10 and 1:20) dilutions of rabbit serum 3 h before saline treatment (ICV) had no effect on the colon temperature, and it did not modify the PACAP-38-induced hyperthermia (Fig. 15).

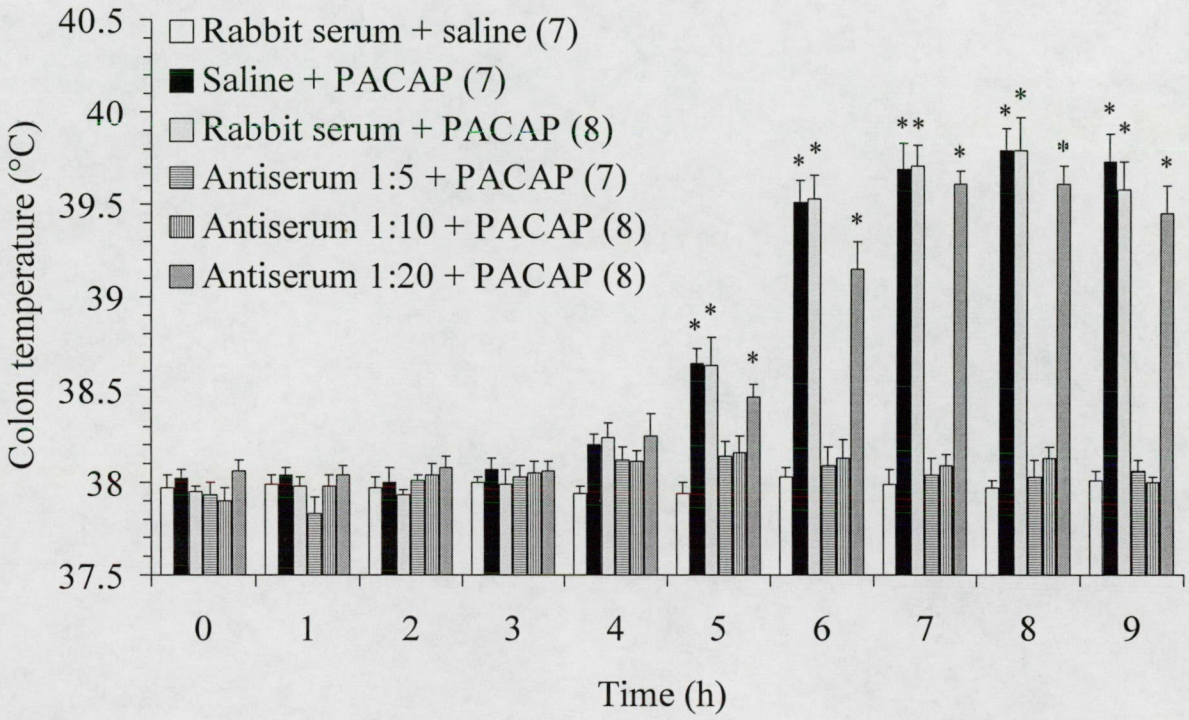

Figure 15

Effects of PACAP-38 antiserum (PACAP-38-AB) on body temperature and on PACAP-induced hyperthermia

Groups of rats received an ICV injection of saline or different dilutions (1:5, 1:10 or 1:20) of either PACAP-38-AB (antiserum + PACAP groups) or normal rabbit serum 3 h before (at 0 h) PACAP-38 (1000 ng, ICV) or saline (ICV) treatment (at 3h). (Groups of animals receiving different dilutions of PACAP-38-AB 3 h before an ICV saline injection are not shown). Number of animals per group is presented in parentheses after the corresponding

group. The vertical lines on the top of the bars denote the S.E.M. * $p < 0.05$, compared with the rabbit serum + saline group.

3.5 The effects of noraminophenazone on the PACAP-38-induced hyperthermia

A 50 mg/kg IM injection of the cyclooxygenase inhibitor noraminophenazone 30 min before peptide administration abolished the hyperthermic effect of 1000 ng PACAP-38 [$F_{1;\,2;\,3;\,4;\,5;\,6\ h}$ (3, 31) = 0.45; 9.97; 44.89; 105.29; 51.31; 56.75; $p < 0.05$]. Noraminophenazone alone in a dose of 50 mg/kg IM did not affect the body temperature (Fig. 16).

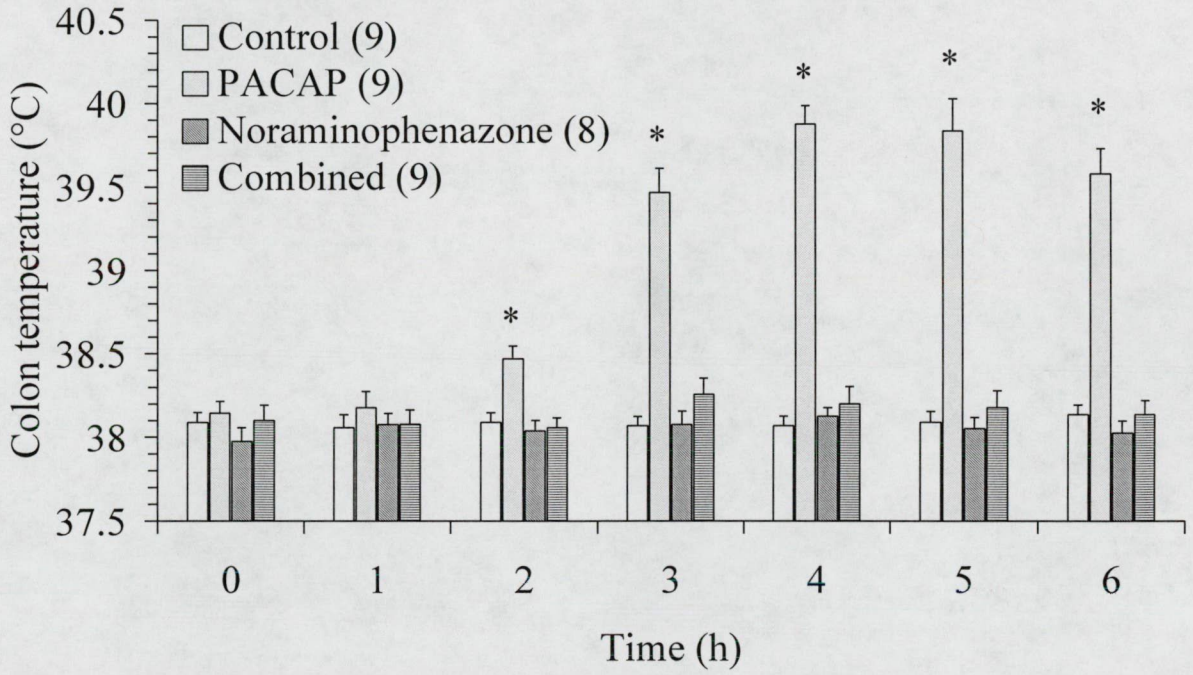

Figure 16

Effects of noraminophenazone on PACAP-38-induced hyperthermia

Groups of rats received an IM injection of saline (PACAP-38 group) or 50 mg noraminophenazone (combined group) 30 min before an ICV injection of 1000 ng PACAP-38. The noraminophenazone group received an IM injection of noraminophenazone (50 mg) 30 min before an ICV saline injection. The control group received an

IM saline injection 30 min before saline treatment (ICV). Number of animals per group is presented in parentheses after the corresponding group. The vertical lines on the top of the bars denote the S.E.M. * $p < 0.05$, compared with the control group.

4 Discussion

Body temperature is regulated and monitored by specific regions of the CNS, and particularly regions of the anterior hypothalamus, which acts as a thermostat by altering the balance of heat loss and production via the sympathetic nervous system. Inflammatory processes in the organism induce certain host responses that are collectively referred to as the acute-phase response. An elevation of body temperature is part of this response. Mediators of this hyperthermia are some of the same mediators that account for other manifestations of the acute-phase response.

Cytokines are pleiotropic molecules mediating a number of pathologic processes. Pyrogenicity is a fundamental biological property of several cytokines, such as IL-1 (27, 41) and IL-6 (39, 58), which stimulate thermogenesis at least partially via the synthesis of prostaglandins (30, 70, 88), however the mechanism by which pyrogens in the circulation promote the appearance of these metabolites of the cyclooxygenase pathway in the brain is not clear (24). Antipyretic drugs reduce fever by blocking the production of specific prostaglandins, and in particular PGE_2, although there are data that support the hypothesis on non-steroid anti-inflammatory drugs (NSAID) producing their antipyresis at least partially via stimulation of AVP release or action (1, 47).

Some endogenously produced peptides have been found to affect the body temperature when administered to experimental animals (22). Those that can be found in the CNS are of overriding importance, since they may be involved in thermoregulatory processes under physiological conditions. Numerous studies have reported on neuropeptides, which can affect body temperature under experimental circumstances, but their physiological importance remains unclear (17, 33, 45, 48, 53, 64, 74, 79, 97).

Since members of the natriuretic peptide family, PACAP and receptors for these peptides are all present in the brain, and it is widely accepted that these neuropeptides have significant roles in neuroendocrine regulation at a central level, in our experiments we investigated the possible participation of PACAP and natriuretic peptides in thermoregulation. Our data connected hyperthermic effects of these peptides to some known mediators of *in vivo* hyperthermic events.

4.1 The effects of ANP-28, BNP-32 and CNP-22 on body temperature

The effects of centrally administered natriuretic peptides on body temperature have been revealed in this series of experiments. Injections of ANP-28, BNP-32 or CNP-22 into the lateral ventricle of the brain elevated the body temperature of the experimental animals. A positive correlation between the hyperthermic effect and the dose of peptide administration could be observed for ANP-28 and CNP-22.

Several authors have investigated the distributions of ANP and related peptides and their receptors in the CNS. The central presence and localization of these peptides and their receptors has been identified, and it is now widely accepted that natriuretic peptides participate in neuroendocrine regulation and also that some of them may act in neuromodulation between glial cells and neurons (57, 104). ANP and CNP-encoding messenger RNA-positive neurons have been successfully detected in the medial preoptic area of the hypothalamus (a brain region that is known to take part in temperature control) in rats (83).

Our results indicate that all members of the natriuretic peptide family cause hyperthermia when administered into the rat brain. Nevertheless, no other data are yet available to demonstrate that natriuretic peptides affect thermoregulatory events in the CNS.

The comparison of the hyperthermic effects of the most efficacious doses of ANP and related peptides showed that the doses of the different types of natriuretic peptides used had similar hyperthermic potency.

Despite that the administered effective doses of ANP and related peptides in our experiments are above the physiological range, the possible participation of a pathway involving natriuretic peptides in hyperthermic events under pathological conditions is not to be precluded. The localization of the site of hyperthermic action in the CNS as well as the route(s) of natriuretic peptides from the cerebroventricle to the active site are both to be identified to make the ICV doses of these peptides comparable to physiological ranges. Although CNP was reported to be the major natriuretic peptide in human cerebrospinal fluid (46), our results suggest that ANP-28 and BNP-32 may also have neuroendocrine actions in the CNS.

4.2 The effects of noraminophenazone on the natriuretic peptide-induced hyperthermia

Possible participations of cyclooxygenase-products in the mediation of natriuretic peptides-induced hyperthermia were tested by the inhibition of the cyclooxygenase-pathway of the arachidonate cascade with a NSAID. The inhibition of prostaglandin production by noraminophenazone - a known inhibitor of prostaglandin synthesis (18) -, completely abolished the hyperthermia induced by ANP and related peptides.

Connections between natriuretic peptides and prostaglandins were recently reported. Effects of ANP and a circulating peptide portion (ANP-31-67) of pro-ANP mediated by the generation of PGE_2 were found in the kidney (38, 44, 105), and ANP, which increases cellular cyclic GMP by activating membrane-bound guanylate cyclase, was suggested to amplify the IL-1β-induced cyclooxygenase-2 mRNA expression (96). Bone endothelial cells have been demonstrated to bear receptors for natriuretic hormones associated with changes in prostaglandin production (28).

Our data establish connections of ANP and related peptides with generally admitted pyrogenic substances - although it is still debatable, in view of a review (51), that prostaglandins themselves are essential for the development of all hyperthermic events. These results ascertain the participation of arachidonate metabolites of the cyclooxygenase-pathway in the mediation of the hyperthermia induced by natriuretic peptides in rats even though we are not aware of any direct evidence on other prostaglandin-mediated natriuretic peptide-effects in the CNS.

4.3 The effects of isatin on the natriuretic peptide-induced hyperthermia

The effects of isatin on centrally administered natriuretic peptide-induced hyperthermia in rats have been revealed in this series of experiments. Injections of ANP-28, BNP-32 or CNP-22 into the lateral cerebroventricle elevated the body temperature of the animals, the doses of the different types of natriuretic peptides used having similar

hyperthermic potency. Isatin in a dose which itself had no effect on the basal body temperature completely abolished the hyperthermia induced by ANP and related peptides lending further support to the view that isatin antagonizes the action of the natriuretic peptides at a central level.

Increasing evidence suggests that connections exist between the endogenous indole isatin and members of the natriuretic peptide system. Investigations undertaken to explore the interactions of isatin with natriuretic peptides *in vivo* have focused on water balance (9) and anxiety (86). Although isatin has been found to counteract some effects of these neuropeptides, to our knowledge no other data are yet available to demonstrate that isatin affects central actions of natriuretic peptides *in vivo*.

Our previous results suggested that the possible participation of natriuretic peptides in thermoregulatory processes might be mediated via a pathway involving cyclooxygenase products (78), but we are not aware of any direct evidence concerning interactions between isatin and prostaglandins. Despite the presented evidence that isatin inhibits the hyperthermic effects of ANP and related peptides, the pathways that mediate the hyperthermic action of natriuretic peptides require further investigation.

In summary, it can be concluded that in rats under experimental circumstances centrally administered natriuretic peptides induce hyperthermia with an early onset and a decline by 90 min. An as yet uncharacterized mechanism that can be inhibited by isatin (probably at the level of natriuretic peptide receptors) and includes cyclooxygenase products is suspected of mediating the hyperthermia induced by ANP and related peptides. Our findings – beyond the view that isatin acts on the ANP receptor, which was based upon *in vitro* results (69) – produce evidences that isatin can also inhibit effects of other members of the natriuretic peptide family *in vivo*.

4.4 The effects of PACAP-38 on body temperature and the effects of PACAP-38-AB on colon temperature and on the PACAP-38-induced hyperthermia

Counteracting effects of ICV administered PACAP-38 and thyrotropin-releasing hormone (TRH) but not VIP on the reserpine-induced hypothermia in murines have recently

been reported (61). An increase in the body temperature of reserpine-injected mice to the normal control level (37.8 ± 0.1 °C) 120 min after administration of the peptide was observed. PACAP was 500 times more potent than TRH in recovering reserpine-induced hypothermia in this study. It was suggested in consequence that PACAP might play an important role in regulating body temperature in the CNS. In spite of the presented evidence that PACAP has a direct action on hypothermia, to our knowledge no other data are yet available to demonstrate that PACAP affects thermoregulatory events in the CNS.

The effects of centrally administered PACAP-38 on body temperature have been revealed in the first series of our experiments. Injections of the peptide into the lateral ventricle of the brain elevated the body temperature of the experimental animals with an onset of the hyperthermia at 2 h and a decline at 6 h. A positive correlation was observed between the hyperthermic effect and the dose of peptide administered.

In order to show the specificity of the hyperthermic action for PACAP-38, we investigated the effects of PACAP-38-AB on PACAP-38-induced hyperthermia. We found that pretreatment with PACAP-38-AB completely prevented the development of hyperthermia in PACAP-38-treated animals, whereas PACAP-38-AB did not modify the body temperature itself. The latter findings do not support an endogenous role of PACAP-38 in the control of body temperature within normal ranges, but show that the hyperthermic effect of PACAP is selective to PACAP-38, and may suggest a participation of PACAP-38 under pathophysiological circumstances.

In our experiments we found that the course of the hyperthermia induced by PACAP-38 differs from those induced by natriuretic peptides considering that PACAP-38 induces hyperthermia with a later onset and a longer duration than natriuretic peptides.

Some investigators demonstrated delayed or atypical neuroendocrine actions of PACAP. On dispersed rat anterior pituitary cells, PACAP-38 was significantly effective (probably through protein kinase-C activation) on the release and synthesis of luteinizing hormone after 4 h. PACAP-38 effects on GH and corticotropin levels (probably via IL-6 production) did not reach significance until 24 h implying different but uncharacterized mechanisms of PACAP action (39). Others suggested a time-dependent paracrine action of PACAP by showing that a prolonged (38 h) static incubation of a total pituitary cell

suspension with PACAP-38 resulted in increased messenger RNA in prolactine and GH cells (99).

On the grounds of the above findings the time characteristics of the PACAP-38-induced hyperthermia raise the possibility of the involvement of time-consuming paracrine actions on different cell types in the hyperthermic effect of PACAP-38.

4.5 The effects of noraminophenazone on the PACAP-38-induced hyperthermia

Connections between PACAP and prostaglandins have been explored in some species. Although increasing concentrations of PACAP-38 are reported to evoke dose-response enhancement of PGE_2 production in the testis of the rat (81), no data on the rat brain are yet available as concerns PACAP directly increasing PGE_2 synthesis.

Our experiments revealed that the inhibition of prostaglandin synthesis with the cyclooxygenase inhibitor noraminophenazone completely abolished the hyperthermia induced by PACAP-38.

Data have recently been presented on the PACAP type I receptor on macrophages probably involved in controlling certain inflammatory responses (87). It was reported that maxadilan, a selective agonist of the PACAP type I receptor, inhibited TNF-α, but increased the IL-6 (a proinflammatory cytokine) and PGE_2 release of LPS-stimulated macrophages. PACAP-38 had the same effects as maxadilan on cytokine production. In another study, PACAP and VIP were found to enhance the secretion of IL-6 both in unstimulated macrophages and in macrophages stimulated with low (1-10 pg/ml) LPS concentrations (60).

An earlier study reported the synergism of PACAP with IL-1 to stimulate IL-6 secretion in rat astrocytes (35). These stimulatory effects of PACAP and IL-1 were similar to that of LPS on IL-6 secretion.

Wang *et al.* (100) presented data on PACAP participating in ocular inflammation in the rabbit. Zhang *et al.* (106) found that the expression of PACAP is upregulated in sensory neurons by inflammation, and it is therefore suggested by the above authors that PACAP is likely to be involved in the inflammatory response.

Taken together, these data on the connections between endogenous pyrogens and PACAP lend support to our findings as regards the effects of PACAP on body temperature and also to the idea that PACAP might be involved in the inflammatory response.

These results reinforce the significance of the participation of PACAP in neuroendocrine regulation. However the hyperthermic doses of the peptide does not suggest a physiological function of PACAP in thermoregulation, the judgment of the required ICV dose of the peptide for a physiological role is hampered by the factors mentioned above (see p. 30). Our study presents direct evidence that this neuropeptide can increase body temperature, and it is possible that in certain pathophysiological processes, which might release PACAP, it can cause fever via a pathway involving cyclooxygenase products.

5 Summary

The main findings of our experiments:

1) Centrally administered ANP-28, BNP-32 and CNP-22 induce hyperthermia in the laboratory rat with a decline of the hyperthermic effect by 90 min after peptide administration. Natriuretic peptides may participate in hyperthermic events within the CNS.

2) The cyclooxygenase inhibitor noraminophenazone completely abolishes the natriuretic peptides-induced hyperthermia. Prostaglandin products of the arachidonate cascade may contribute to the mediation of the hyperthermic effects of natriuretic peptides in the rat.

3) The endogenous indole isatin – probably by acting on natriuretic peptide receptors – abolishes the natriuretic peptides-induced hyperthermia in the rat. Receptors, which can be inhibited with isatin, are expected to mediate the hyperthermic effects of natriuretic peptides.

4) PACAP-38, when administered centrally, induces hyperthermia in rats and may play a role in hyperthermic events in pathophysiological processes. The time-course of the hyperthermia suggests time-consuming actions in the mediation of the hyperthermic effect of PACAP-38.

5) The inhibition of the cyclooxygenase pathway of the arachidonate cascade abolishes the PACAP-38-induced hyperthermia in rats. Prostaglandin products are suggested to participate in the mediation of the hyperthermia induced by PACAP-38.

Our experiments were designed to investigate the actions of neuropeptides (ANP-28, BNP-32, CNP-22, PACAP-38) on the central control of body temperature and to characterize the mediations of their achievable thermoregulatory effects in rats.

We demonstrated that ANP-28, BNP-32, CNP-22 and PACAP-38 can cause hyperthermia via pathways that involve cyclooxygenase products, and establish the inhibitory effects of isatin on *in vivo* actions of natriuretic peptides. Our results suggest that the examined neuropeptides may participate in hyperthermic events under at least pathophysiological circumstances and may contribute to the neuroendocrine regulation of body temperature.

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